PATENT
Docket No.: 176/61442 (1196)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Miller et al.	)	Examiner: Sarae Bausch
Serial No.	:	10/541,044	)	Art Unit: 1634
Cnfrm. No.	:	1984	)	
Filed	:	January 1, 2004	)	
For	:	HYBRIDIZATION-BASED BIOSENSOR CONTAINING HAIRPIN PROBES AND USE THEREOF	)	

REQUEST FOR RECONSIDERATION

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the July 24, 2009, office action, applicants respectfully request reconsideration of the rejections set forth therein in view of the responses provided below.

No claims amendments have been made. Claims 1-25, 27-37, 42, 44-61, 67, and 68 remain pending, with claims 29-36 and 44-61 standing withdrawn. Claims 1-25, 27, 28, 37, 42, 67, and 68 are under examination.

This submission is accompanied by a petition for a one-month extension of time. All fees associated therewith should be charged to deposit account 14-1138. Any overpayment or underpayment should be applied to this same account.

This submission is also accompanied by the Declaration of Benjamin L. Miller under 37 C.F.R. § 1.132 ("Miller Declaration"). The Miller Declaration, because it is directly responsive to a rejection made for the first time in the outstanding office action, should be entered and considered despite the claims being finally rejected.

The rejection of claims 1-11, 14-21, 24, 27, 28, 67, and 68 under 35 U.S.C. § 103(a) for obviousness over U.S. Patent No. 6,312,906 to Cass et al. ("Cass") in view of Herne

et al., "Characterization of DNA Probes Immobilized on Gold Surfaces," *J Am Chem Soc* 119:8916-8920 (1997) ("Herne") is respectfully traversed. Applicants respectfully disagree for the reasons set forth *infra* and in the accompanying Miller Declaration.

Cass generally teaches the use of surface bound *hairpin* nucleic acids for detection of target nucleic acids. While Cass describes the use of dithiols to control hairpin density, Cass provides no guidance for their use and none of the Examples illustrate their use. Thus, Cass fails to teach or suggest the binding of disclosed dithiols and *hairpin* molecules "following exposure of the fluorescence quenching surface to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater" as recited in claim 1. Cass, therefore, fails to teach or suggest the critical need for the recited ratio of spacer to *hairpin* nucleic acid molecule with respect to the recited fold-increase in fluorescence.

The PTO at page 4 of the office action asserts that Herne overcomes this deficiency of Cass. Applicants respectfully disagree for several reasons.

Firstly, the schematic illustrated in Figure 4B of Herne merely shows a cartoon of the Herne substrate having a 5:1 ratio of MCH:DNA, but this does not overcome the deficiencies of Cass, because Herne never exposes the fluorescence quenching surface to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater. The claim language does *not* recite that a ratio of spacer:nucleic acid of about 5:1 or greater exists on the substrate, but rather that the substrate is exposed to such a mixture.

Secondly, the combination of Cass and Herne would not have arrived at the claimed invention and one of ordinary skill in the art would not have expected as much.

Herne teaches a two step method of controlling the surface coverage of thiol-derivatized *linear* DNA probes on the surface of gold substrate. In the abstract Herne states, "[t]he primary advantage of using this two step process to form HS-ssDNA/MCH mixed monolayers is that nonspecifically adsorbed DNA is largely removed from the surface." Applicants respectfully submit that Herne merely teaches a method of removing excess, nonspecifically bound DNA probes from the surface of the substrate. There is a significant difference between the dissociation kinetics involved in removing nonspecifically adsorbed DNA and the binding kinetics of attachment of DNA probes to a substrate. This difference is evident when these two events are compared in terms of probe distribution on the surface of the substrate.

The first step of Herne involves immersing the substrate in a solution of *linear* single stranded derivatized DNA probes and allowing the *linear* probes to bind to the substrate randomly, either via the thiol or by non specific adsorption. *Only* the total amount (specific + non specific) of DNA probe which gets attached to the surface is controlled by timed exposures. In other words, this binding event fails to control the ratio between the amount of thiol bound probe to the amount of non-specifically adsorbed probe. The spacer thiol, 6-mercapto-1-hexanol (MCH) is subsequently added in the second step and functions to remove the non-specifically adsorbed DNA and to fill in the gaps left by non-specifically adsorbed DNA. In terms of reaction kinetics, the subsequent removal of non-specifically adsorbed DNA probe from the surface would not lead to redistribution of thiol bound probes on the surface. Depending upon a number of other factors, such as ionic concentration, length of DNA probes, temperature, amount of purine versus pyrimidine residues in the DNA probe the binding process could be dominated either by thiol bound DNA or by nonspecific adsorption of the DNA, and could result in, for example, variations in localized concentrations of thiol bound DNA probe. Nevertheless, the two step process described by Herne does not involve exposing the surface to the recited mixture of agents.

Also, it is well known in the art that the folding and self assembly properties of *hairpin* DNA are very different from those of *linear* DNA probes. A person skilled in the art would not be motivated to combine the teachings of Cass and Herne because of these differences. The single stranded *linear* DNA probe has exposed aromatic rings which are extremely hydrophobic and energetically favor reduced exposure of aromatic rings. Such hydrophobic interactions would force the *linear* probes to adsorb to the gold surface much more readily. On the contrary, the *hairpin* molecule is a partially double stranded molecule and would not be exposed to similar hydrophobic constraints. Also, the charge density for a collection of hairpin molecules is more than linear DNA probes, because of the double stranded nature of the hairpin, and would therefore behave differently under similar ionic conditions. Since the hairpin molecules of the present invention require unfolding in order to bind to the target molecule the spacing requirements for a *hairpin* when compared to a *linear* DNA probe would be significantly different. Consequently, one of skill in the art would have expected that the method of Herne would not work with hairpin probes.

The accompanying Miller Declaration provides evidence that an expectation of success would have been lacking and that Herne does not overcome the deficiencies of Cass.

Specifically, the Miller Declaration at ¶¶4-7 demonstrates that a sensor chip synthesized by using the optimized synthesis steps of Herne with hairpin probes (i.e., as described by Cass) does not afford a functional sensor chip. In contrast, a sensor chip prepared in accordance with the presently claimed invention results in a sensor chip that exhibits at least a 5-fold increase in fluorescence intensity upon exposure to target nucleic acid molecules. The exemplary sensor chip prepared for comparison using the same hairpin and target—but instead exposing the chip to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater, as claimed—resulted in a chip that exhibited a 20-fold increase in fluorescence intensity (*see* Miller Declaration, ¶ 6). For this reason, one of skill in the art would not have had a reasonable expectation of success in combining the teachings of Cass and Herne to arrive at the claimed invention. Moreover, the combination of Cass and Herne does not teach each and every aspect of the claimed invention because of these deficiencies.

For these reasons, the rejection of claims 1-11, 14-21, 24, 27, 28, 67, and 68 for obviousness over the combination of Cass and Herne is improper and should be withdrawn.

The rejection of claims 12, 13, 22, and 23 under 35 U.S.C. § 103(a) for obviousness over the combination of Cass and Herne, and further in view of U.S. Published Patent Application No. 2002/0034747 to Bruchez et al. ("Bruchez") is respectfully traversed.

The teachings and deficiencies of the combination of Cass and Herne with respect to claim 1 are noted above.

Bruchez is cited at page 6 of the office action for teaching use of semiconductor nanocrystal labels attached to different polynucleotides for simultaneous analysis. The PTO has failed to demonstrate how Bruchez overcomes the above-noted deficiencies of Cass and Herne. For this reason, the rejection of claims 12, 13, 22, and 23 for obviousness over the combination of Cass, Herne, and Bruchez is improper and should be withdrawn.

The rejection of claims 37 and 42 under 35 U.S.C. § 103(a) for obviousness over Cass in view of Vannuffel et al., "Specific Detection of Methicillin-Resistant *Staphylococcus* Species by Multiplex PCR," *J. Clin. Microbiology* 33(11):2864-2867 (1995) ("Vannuffel"), Berger-Bachi et al., Genbank accession No. X17688 ("Berger-Bachi") and U.S. Patent No. 5,541,308 to Hogan et al. ("Hogan") is respectfully traversed.

The teachings of Cass and noted above, and the PTO correctly notes that Cass does not teach SEQ ID NO: 1 (or any of SEQ ID NOS: 2-10 or 13, for that matter).

Vannuffel teaches detection of *femA* gene of methicillin-resistant Staphylococcus species by using two different oligonucleotide probes that are complementary to PCR-amplified regions of *femA*. Neither of these probes is a hairpin probe. Berger-Bachi teaches the entire sequence of the *femA* gene. Hogan teaches a method of preparing oligonucleotides that are sufficiently complementary to a unique region of rRNA for a hybridization assay. The PTO asserts at pages 7-10 of the office action that it would have been obvious to a person of ordinary skill in the art to use the *femA* sequence taught by Berger-Bachi and Vannuffel to design *femA*-specific probes as taught by Hogan and use such probes in a manner taught by Cass. Applicants respectfully disagree for several reasons.

Firstly, Hogan would not have been combined with the other cited references, particularly Cass, because Hogan explicitly instructs (in the language quoted by the PTO) that "probes with extensive self-complementarity should be avoided." Hogan, therefore, would have taught away from using hairpin probes.

Secondly, SEQ ID NO: 1 includes 48 nucleotides, of which—as noted by the PTO—only nt 10-41 will hybridize with *femA*. In other words, the portion of SEQ ID NO: 1 that hybridizes contains 32 contiguous nucleotides of *femA*. In consideration of just the 1302 nt coding sequence of *femA*, there would be 1270 distinct 32 nt probes that could be obtained for *femA*. SEQ ID NO: 1 also includes a specific 9 nt 5' sequence and a specific 7 nt 3' sequence. There are 16,384 possible 7-mers to choose from, and the 5' 9-mer region was selected to base pair with nt 40-48. Thus, in consideration of the 1270 distinct *femA* 32-mers and 16,384 potential 7-mers, over 20.8 million possible 48 mer constructions exist.

Applicants are claiming but one of these >20.8 million possibilities, and nowhere is this specific combination of nucleotides taught or suggested by the combination of references. Therefore, the rejection of claims 37 and 42 over the combination of Cass, Vannuffel, Berger-Biachi, and Hogan is improper and should be withdrawn.

The rejection of claim 25 under 35 U.S.C. § 103(a) for obviousness over Cass in view of Herne and further in view of Vannuffel, Berger-Bachi, and Hogan is respectfully traversed.

The teachings and deficiencies of the combination of Cass and Herne are noted above. Moreover, SEQ ID NO: 1 would not have been obvious for the reasons noted above. Because the PTO has failed to demonstrate how Vannuffel, Berger-Bachi and Hogan overcome these deficiencies, the rejection of claim 25 for obviousness over the combination of Cass and

Herne and further in view of Vannuffel, Berger-Bachi, and Hogan is improper and should be withdrawn.

The provisional rejection of claims 1-25, 27, 28, 37, 42, 67, and 68 on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-14 of copending U.S. Patent Application Serial No. 11/838,616 to Miller et al. ("Miller I") is respectfully traversed. Although applicants submit that the claims of the later-filed application are patentably distinct, withdrawal of this provisional rejection is nevertheless proper pursuant to *Manual of Patent Examining Procedure* § 804 (p. 800-17). Because all other rejections should be withdrawn for the reasons discussed (*supra* and *infra*), the provisional obviousness-type double patenting rejection of claims 1-25, 27, 28, 37, 42, 67, and 68 over claims of Miller I should be withdrawn.

The rejection of claims 1-25, 27, 28, 37, 42, 67, and 68 on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 1-8, 11, 13, 14, and 17-21 of U.S. Patent No. 7,442,510 to Miller et al. ("Miller II") is respectfully traversed in view of the Terminal Disclaimer filed herewith. This rejection should be withdrawn.

The rejection of claims 37 and 42 on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 1-8, 11, 13, 14, and 17-21 of Miller II in view of Cass and Herne is improper. SEQ ID No: 1 does not hybridize over its full length to *femA* for these reasons noted above. Claims 1 and 17 of Miller II both require hybridization over the full length to a target nucleic acid that has a naturally occurring sequence. Because SEQ ID No: 1 lacks this feature, the rejection is improper. Nevertheless, in view of the Terminal Disclaimer filed herewith, this rejection should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: November 23, 2009

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